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Studies on Anti-tuberculous Low Molecular Factors in Various Organs of Rabbits

Part II. Studies on fractionated purified materials using ion exchange resins.

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INTRODUCTION

In Part I, the presence of tuberculostatic substances in the crude materials of rabbit's organ extracts was reported. The active substances are able to pass through a cellophane membrane, heat stable and soluble in methyl alcohol but insoluble in ether. As it seems possible that the crude materials of rabbit's organ extracts may contain many other substances besides the active factors, it may be desirable to remove the mixed inactive factors and to purify the active factors. For this purpose, the procedures which have been reported by Oshima or Fujita[1)2)3)], that is, the fractionation procedures using ion exchange resins, were used.

The investigations dealing with the fractionation procedures, the fractionated materials, their antimycobacterial activities and chemical characteristics will be reported in this paper.

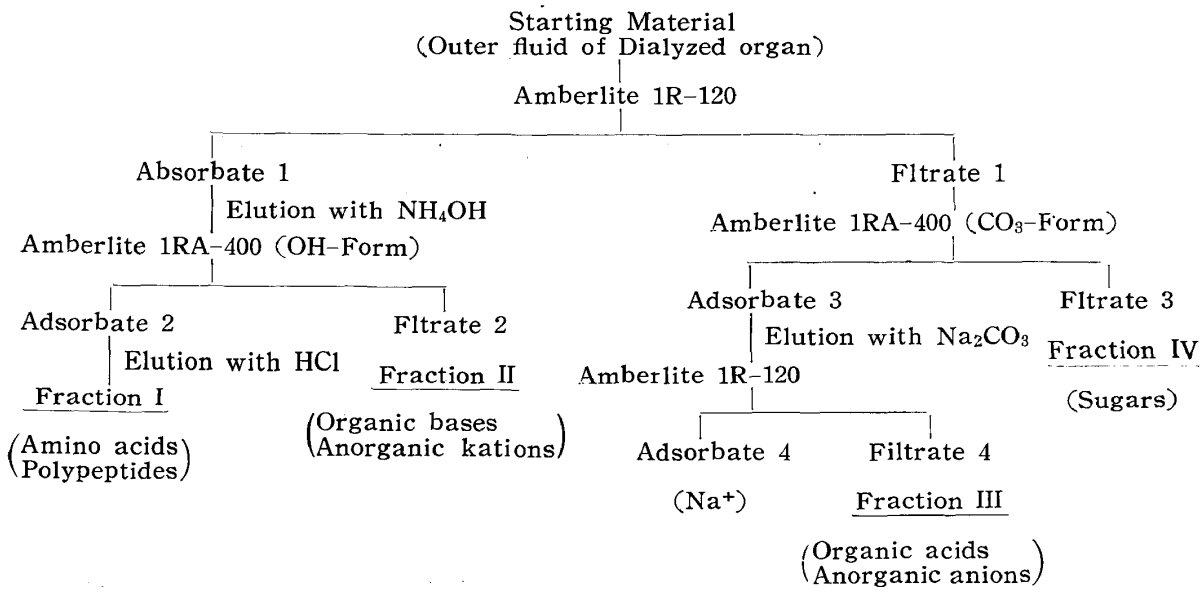
MATERIALS AND METHODS

As starting materials, dialyzed outer fluid of organ extracts from muscle, liver, kidney and lung of normal or immunized rabbits were prepared as described in Part I. In order to obtain enough material to fractionate, four rabbits were used.

Technique of chemical fractionation of low molecular substances in rabbit's organ extract :

The adsorption technique was used, using ion exchange resins. As the technique was reported in a previous paper[1)] in detail, only a diagram is presented in Table 1 in this paper. As shown in the table, the low molecular substances of rabbit's organ extract were separated into four fractions as follows:

Table 1. Procedures used in chemical fractionation of low moleculal substances in organ extracts of rabbits.



Fraction I : adsorbed both on cation and antion exchange resins (amino acids, peptides)

Fraction II : adsorbed on cation but not adsorbed on anion exchange resins (organic bases)

Fraction III : adsorbed on anion but not adsorbed on cation exchange resins (organic acids)

Fraction IV : adsorbed on neither cation nor anion exchange resins (sugars etc.)

Each fraction was concentrated to dryness under reduced pressure and low temperature (at 10 mg Hg 50°C), was weighed, and dissolved in water of 1/8 the weight of the original organ. Thus, an eight-fold concentrated solution of each fraction was obtained.

Test for tuberculostatic activity :

By the same procedure as described in Part I, the tuberculostatic activity of each fraction was tested.

As Fractions I and III were strongly acidic (pH under 1.2), they were tested for their tuberculostatic activity after being neutralized with NaOH to pH 7.0.

As test bacteria, H37Rv strain, Bovine Rm strain and Avian Cho-kyo strain were used.

Paper Chromatgraphy :

Chemical analysis of each fraction was carried out by using the paper chromatography as follows :

Methods of developing ; Ascending method (In the present investigations,

mainly one-dimension method was used) and sometimes circular chromatography was used.

Solvents for developing ; n-buthanol-acetic acid-water (4 : 1 : 2) was mostly used to delope each fraction. Besides, for Fraction III, ether-acetic acid-water (13 : 3 : 1), n-buthanol-methanol (1 : 1) or n-buthanol-formic acid-water (10 : 2 : 15) were used.

Detection ; Ninhydrin reaction (for Fraction I) Jaffe's reaction (for Fraction II) ammoniac silver nitrate (for Fraction III and IV), fluolescence by ultraviolet ray or pH indicator (for Fraction III) were used.

RESULTS

1. The inhibitory effect of the fractionated materials of several organ extracts of rabbits on the growth of H37Rv strain.

i) Fractionated materials from normal rabbits : The results are shown in Table 2.

Table 2. The effect of the fractionated materials of several organ extracts from normal rabbits on the growth of tubercle bacilli.

Organs, Fractions		Concentration					
		8×	4×	2×	1×	1/2×	1/4×
Muscle	I	−	−	+	+++	+++	+++
	II	+++	+++	+++	+++	+++	+++
	III	−	−	+	++	+++	+++
	IV	+++	+++	+++	+++	+++	+++
Liver	I	−	−	−	+	+++	+++
	II	++	+++	+++	+++	+++	+++
	III	++	+++	+++	+++	+++	+++
	IV	−	+	+++	+++	+++	+++
Kidney	I	−	−	−	++	+++	+++
	II	+++	+++	+++	+++	+++	+++
	III	++	+++	+++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++
Lung	I	−	+	++	+++	+++	+++
	II	+++	+++	+++	+++	+++	+++
	III	−	−	+++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++

Control : +++

Slide culture using H37Rv in Kinchner's medium for ten days.

Muscle ; Fractions I and III could inhibit the growth of H37Rv in a concentration of "4×" (four times the concentration of the starting organs), but Fractions II and IV could not inhibit growth even at "8×" concentration.

Liver ; Fraction I could inhibit the growth of H37Rv at "1×" concentration (the same concentration as the starting organs), but Fractions II and III permitted the growth of H37Rv. It may be worthy of note that Fraction IV could in-

hibit the growth of H37Rv at as high concentrations as "8 ×".

Kidney; The results were the same as obtained in the liver but Fraction IV could not inhibit the growth of H37Rv.

Lung ; Although no inhibitive activity with the crude materials at "2×" concentration was noted in Part I, Fraction I could inhibit the growth of H37Rv at "8×" concentration, and Fraction III at "4×". The Fractions II and IV could not inhibit the growth of H37Rv.

The titer of the tuberculostatic activity of the muscle was lower in the fractionated materials than in the crude materials,.....the crude materials could inhibit the growth of H37Rv at as low concentration as "1×", but the fractionated materials could not inhibit growth even at a concentration of "2×"..... On the other hand, in the liver and kidney, Fraction I could inhibit the growth of H37Rv strain at the same concentration as the crude material, that is "1×".

The real activity in mg per ml of the active substances was calculated from Table 2 and the weight of

Table 3. The amount of the fractionated materials of organ extracts of rabbits and their effect on the growth of tubercle bacilli.

Organ	Fraction	Amount of materials mg/ml												
		34	32	30	28	26	24	22	20	18	16	14	12	10	8	6
Muscle	I	−	−	−	−	−	−	−					+	+	+	+
	II										+	+	+	+	+	+
	III	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+
	IV										+	+	+	+	+	+
Liver	I	−	−	−	−	−	−	−	−	−	−				+	+
	II															+
	III	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+
	IV										+	+	+	+	+	+
Kidney	I	−	−	−	−	−	−				+				+	+
	II															+
	III	−	−	−	−	−	−		−	−	+	+	+	+	+	+
	IV										+	+	+	+	+	+
Lung	I	−	−	−	−	−	−	−	−	−				+	+	+
	II															+
	III	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+
	IV										+	+	+	+	+	+

(This was calculated from the result of Table 2.)

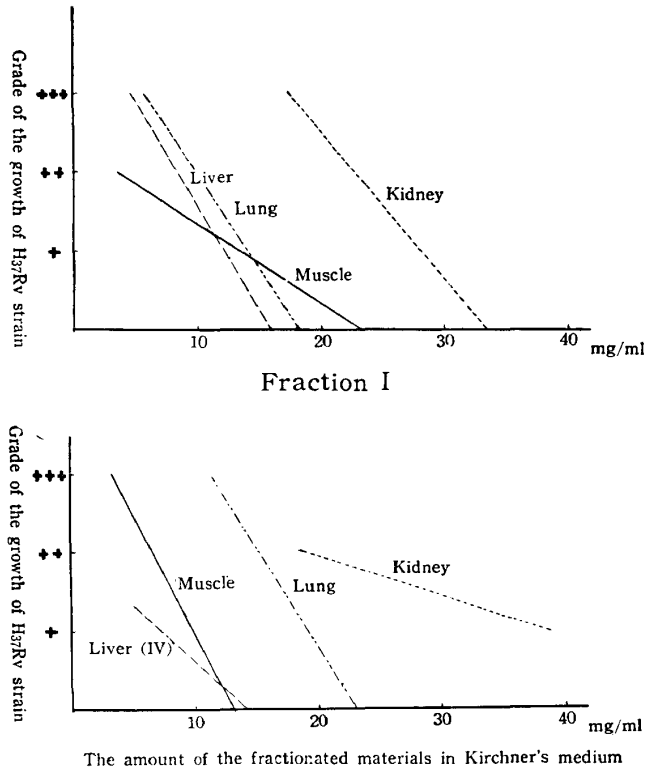


Fig. 1. The effect of the fractionated materials of the organ extracts from normal rabbits on the growth of H37Rv strain.

the fractionated materials, and the results are shown in Table 3 and Fig. 1. Fraction I could inhibit the growth of H37Rv at 22 mg/ml in the muscle, 16 mg/ml in the liver, 32 mg/ml in the kidney, and 18 mg/ml in the lung. Fraction III could inhibit at 12 mg/ml in the muscle, 23 mg/ml in the lung, and Fraction IV could inhibit at 14 mg/ml in the liver.

ii) Fractionated materials from immunized rabbits : The results are shown in Table 4.

No distinct difference between the results obtained from normal rabbits and those from immunized ones are noted. Although the titer of activity in Fraction I of the liver and the lung are higher by one column of concentration in the immune animal than that in the normal

Table 4. The effect of the fractionated materials of several organ extracts from the immunized raddits on the growth of tubercle bacilli

Organ	Fraction	Concentration 8×	4×	2×	1×	1/2×	1/4×
Muscle	I	−	−	−	++	+++	+++
	II	+++	+++	+++	+++	+++	+++
	III	−	−	++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++
Liver	I	−	−	−	−	++	+++
	II	+	+++	+++	+++	+++	+++
	III	++	+++	+++	+++	+++	+++
	IV	−	+	+++	+++	+++	+++
Kidney	I	−	−	−	+++	+++	+++
	II	+++	+++	+++	+++	+++	+++
	III	+	++	+++	+++	+++	+++
	IV	+	++	+++	+++	+++	+++
Lung	I	−	−	++	++	+++	+++
	II	++	+++	+++	+++	+++	+++
	III	−	−	+++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++

Control +++

Slide culture using H37Rv in Kirchner's medium for ten days.

rabbit, the difference is so small that it cannot be thought to be the result of immunity.

2. Inhibitory effect of the fractionated materials of the muscle and the liver of rabbits on the growth of Bovine type and Avian type tubercle bacilli.

Fraction, I, both of the muscle and the liver, could inhibit the growth both of Bovine type (Bovine Rm) and Avian type (Avian Cho-kyo) of tubercle bacilli as Human type (H37Rv) (Table 5). Fraction III was more active in inhibiting

Table 5. The effect of the fractionated materials of muscle and liver of rabbits on the growth of the bovine and avian type of tubercle bacilli.

Organ Fraction / Type of Bacilli		Concentration					
		8×	4×	2×	1×	1/2×	1/4×
Muscle	I	−	−	−	+++	+++	+++
	II	+	+++	+++	+++	+++	+++
Bovine Type	III	−	−	−	−	++	+++
	IV	+	+	+++	+++	+++	+++
Muscle	I	−	−	−	+++	+++	+++
	II	+++	+++	+++	+++	+++	+++
Avian Type	III	−	+++	+++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++
Liver	I	−	−	−	+	+++	+++
	II	++	+++	+++	+++	+++	+++
Bovine Type	III	−	−	+	+++	+++	+++
	IV	+	+++	+++	+++	+++	+++
Liver	I	−	−	−	++	++	++
	II	+++	+++	+++	+++	+++	+++
Avian Type	III	−	++	+++	+++	+++	+++
	IV	+++	+++	+++	+++	+++	+++

Control : Bovine type +++, Avian type +++
Slide culture in Kirchner's medium for ten days.

the growth of Bovine Rm than that of H37Rv, that is Fraction III of the muscle could inhibit the growth of Bovine Rm even at a concentration of "1×", while it could hardly inhibit the growth of H37Rv at a concentration of "4×", and Fraction III of the liver which could not inhibit the growth of H37Rv, could inhibit Bovine Rm at a concentration of "4×". On the other hand, Fraction III was less inhibitory to the growth of the Avian type than to that of the Bovine type. It can only slightly inhibit the growth of Avian Cho-kyo at the high concentration of "8×". Fraction IV of the liver could not inhibit the growth of either type of tubercle bacilli.

3. Effect of hydrolysis on the tuberculostatic activity of the active fractions.

As shown in Table 6, there were no distinct changes in tuberculostatic active fractions (Fraction I of the muscle, the liver and the kidney, Fraction III of the muscle), after being hydrolysed by heating in boiling water with 6N HCl

Table 6. The effect of hydrolysis on the tuberculostatic activity of the active fractions of muscle, liver and kidney of rabbits.
Hydrolysis was carries out by heating in boiling water with 6N HCl for 15 hours.

Organ	Fraction	Hydrolysis	Concentration 8×	4×	2×	1×	1/2×	1/4×
Muscle	I	Before	−	−	+	+++	+++	+++
		After	−	−	+	++	+++	+++
	III	Before	−	−	+	+++	+++	+++
		After	−	−	++	++	+++	+++
Liver	I	Before	−	−	−	+	+++	+++
		After	−	−	−	++	+++	+++
Kidney	I	Before	−	−	−	+++	+++	+++
		After	−	−	−	+++	+++	+++

Cotrol +++
Slide culture using H37Rv in Kirchner's medium for ten days.

for 15 hours. It may be assumed that the active fractions are very stable to hydrolysis.

4. Nature of the activity of the active fractions of tubercle bacilli.

To examine whether the tuberclo-inhibitory activity of the active fractions was tuberculocidal or tuberclostatic, a series of test tubes, in which 2 ml of solution of the active fraction serially diluted with distilled water were contained, were prepared. Two slides on which the tubercle bacilli (H37Rv) were smeared by the benzine method were immersed in a test tube, and incubated at 37°C. One of them was taken out after 24 hours and other after 48 hours. The slides taken out from the incubator were transferred into 2 ml of ordinary Kirchner's serum medium for cultivating for 10 days. The results are shown in Table 7. It can be noted that tubercle bacilli which were immersed in the solution of ac-

Table 7. Nature of the activity of the active fractions (muscle and liver) on the growth of tubercle bacilli.

Organ	Fraction	Immersed Period	Concentration 8×	4×	2×	1×	1/2×	1/4×	Control
Muscle	I	24 hours	−	−	++	+++	+++	+++	+++
		48 hours	−	−	−	+++	+++	+++	+++
	III	24 hours	−	−	++	+++	+++	+++	+++
		48 hours	−	−	++	+++	+++	+++	+++
Liver	I	24 hours	−	−	−	+	+++	+++	+++
		48 hours	−	−	−	+	+++	+++	+++

The bacilli (H37Rv strain) were immersed in the solution of active fractions for 24 or 48 hours, then the bacilli were tranferred into ordinary Kirchner's serum medium and caltivated for ten days. As the control, the physiologic saline solution was used.

tive fractions at high concentration for 24 or 48 hours were already incapable of growth when transfrred to the test tube containing Kirchner's medium. It seems that the active fractions can act bactericidaly on the tubercle bacilli.

5. Chemical investigations of each fraction.

The chemical characteristics of each fraction were examined. In order to confirm the assumption (in Table 1.), some qualitative tests were carried out. Results are shown in Table 8. From these results, it can be assumed that the

Table 8. The chemical characteristics of each fraction.

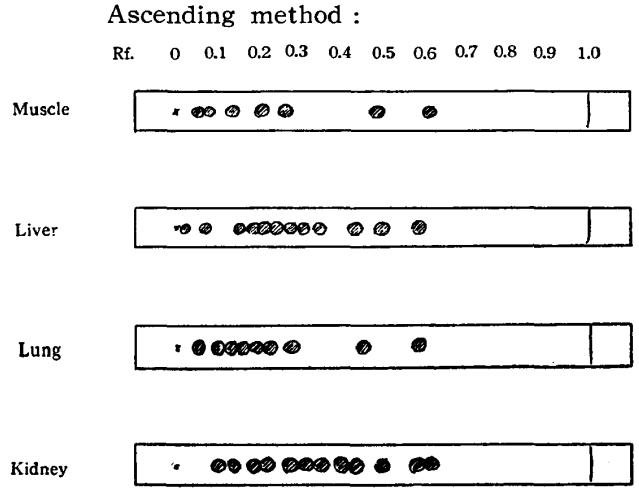
Reaction	Fraction			
	I	II	III	IV
Sulfosalicilic acid	−	−	−	−
Ninhydrin	++	±	±	−
Molisch's R.	−	−	−	++
Jaffé's R.	−	+	−	−
pH	<1.2	>8.0	<1.2	7.0

materials contain no protein and each fraction consists of substance which have been assumed to be from Table 1.

The cheical analysis of each fraction using paper chromatography was as follows :

Fraction I :

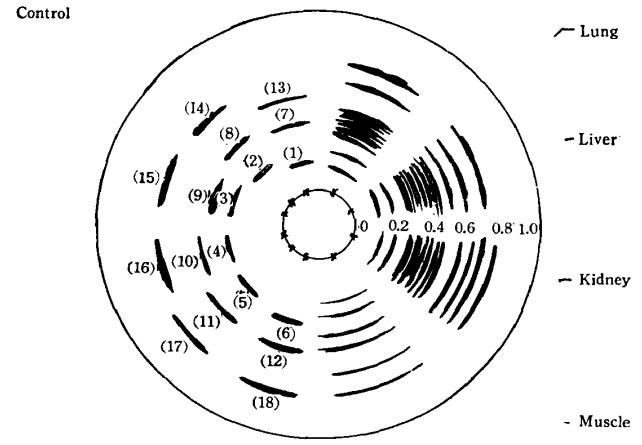
The dried material was colored yellowish-brown like caramel and the aqueous solution of this material was strongly acidic (under pH 1.2). When neutralized with NaOH, a flaky precipitation occurred. This precipitation appeared only under neutral conditions (pH between 5.0-8.0) and concentrated conditions (above 4-fold). The separation of the precipitate from the supernatant failed for technical reasons. Identification by paper chromatography was done with mixed materials. The chromatographic patterns of Fraction I are shown in Figs. 2 and 3.



Developer: n-buthanol-acetic acid-water (4 : 1 : 2)
Indicator: ninhydrine

Fig. 2. The chromatogram of Fraction I of muscle, liver, lung and kidney of rabbits.

Muscle : As shown in Fig. 2, six or seven spots were detected in Fraction I of muscle. It can be as-



Developer: n-buthanol-acetic acid-water (4 : 1 : 2)
Control: amino acids

(1) Cystine	(10) Threonine
(2) Lysine	(11) Aldnine
(3) Arginine	(12) Proline
(4) Histidine	(13) Tyrosine
(5) Asparatic acid	(14) Methionine
(6) Serine	(15) Valine
(7) Oxiproline	(16) Tryptophane
(8) Glutamic acid	(17) Phenylalanine
(9) Glycine	(18) Leucine

Fig. 3. Circular chromatogram of Fraction I of muscle, liver, kidney and lung.

thionine and leucine.

Kidney : It was believed that there were 11 or 12 spots which were similar to the spots in the liver.

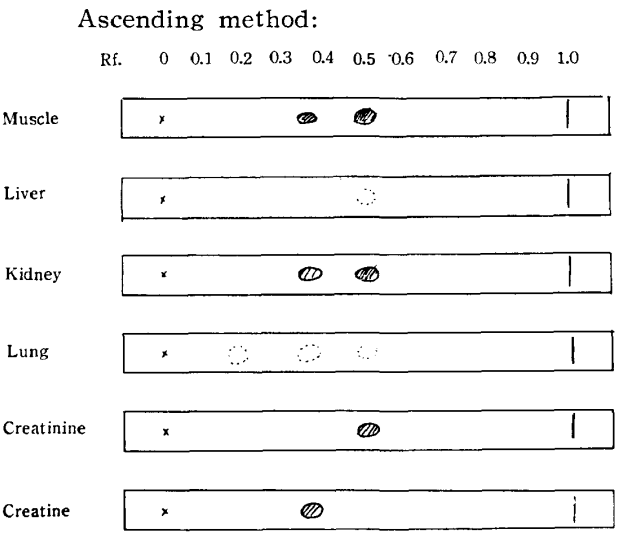
Lung : Nine spots were detected.

Fraction II

This fraction consists of organic or inogranic bases. Fraction II of the muscle could be obtained in the form of white crystaline powder. The Fraction II of other organs could not be obtained in powder but in the form of white or non-colored amorphous substances. The yield of this fraction was the smallest among the four fractions.

It can be assumed that, as sumed that they are histidine, arginine, glycine, threonine, alanine, methionine or valine and leucine in the order of increasing Rf. It was worthy of note that histidine appeared in the muscle, while it could not be found in the other organs.

Liver : More spots (about 12) occurred than in muscle. The spots between Rf. 0.1 and Rf. 0.24, however, were not well separated. Therefore, it was impossible to determine exactly what the spots were which were not present in the muscle. But it is probable that they are cystine and lysine (Rf. smaller than histidine) and phenylalanine (or tryptophan) located between me-



Indicator: Jaffe's reagent
Control: Creatinine (directly)
Creatine (after heating at 100°C for 1 hours)
Developer: n-buthanol-acetic acid-water (4 : 1 : 2)

Fig. 4. The chromatogram of Fraction II of muscle, liver, kiclney and lund of rabbits.

shown in Fig. 4, although there may be some other substances, it is almost certain that in the muscle and the kidney, Fraction II contains creatinine and creatine as its main components, when tested by chromatography. There appeared to be neither creatinine nor creatine in the liver or the lung.

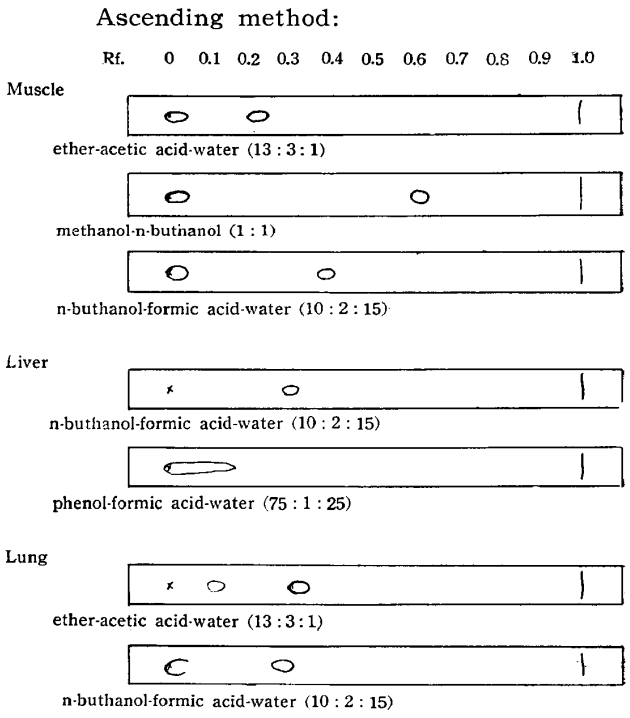
Fraction III :

This is the fraction consisting of organic acids. The detection of the components of Fraction III was not so easy as of Fraction I. In general, as indicators of acids, in chromatography, ammoniac silver nitrate, pH indicator, fluorescence by ultraviolet ray, etc. are used. Fig. 5 shows that the muscle Fraction III always showed two spote against the ammoniac silver nitrate solution regardless of the sort of developer used and the spots coincided with the fluerescent spots detected by ultraviolet ray.

In the lung, also, two spots were detected but they were not the same as in the muscle.

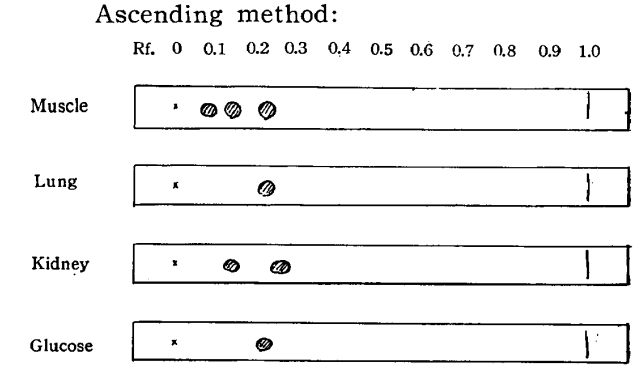
Fraction IV :

This is the fraction containing "sugars". The monosaccarides can be detected by ammoniac silver nitrate solution in paper chromatography. As the developer, n-butanol-acetic acid-water was used, and the results obtained are shown in Fig. 6. There are three spots in the muscle, two spots in the kidney but only one in the liver. The spots appearing near the Rf 0.2 are thought to be glucose.



Developer:
Indicator: ammoniac silver nitrate solution or fluorescence of ultraviolet ray

Fig. 5. The chromatogram of Fraction III of muscle, liver, lung of rabbits.



Developer: n-buthanol-acetic acid-water (4 : 1 : 2)
Indicator: ammoniac silver nitrate solution

Fig. 6. The chromatogram of Fraction IV of muscle, liver and kidney of rabbits.

DISCUSSION

Several investigators have re-

ported on antituberculous factors in animal body fluid.

Dubos[4)5)] reported that there was an antituberculous substance which was extracted from the kidneys of guinea pigs with ethanol. It was soluble in ethanol and insoluble in acetone. This substance inhibited the growth of virulent tubercle bacilli but was inactive against avirulent acid fast bacilli. It was identified as spermin or spermidin, a sort of organic base or amine. He also reported that a sort of peptide which was obtained from calf thymus possessed a definite antimycobacterial activity *in vitro*[6)]. The thymus peptide could also be extracted from calf spleen, sheep thymus, beef lymphnode and calf pancreas but not from calf lung and calf liver.

Endo[7)] also obtained a similar peptide to Dubos's thymus peptide by using the same procedure in beef.

Myrvik[8)] investigated a tuberculostatic substance posessing lysozyme-like properties in the serum of immune rabbits. He also assumed that ascorbic acid might be the antituberculous factor in human urine[9)]. But this was denied by Björnesjö later[10)].

Patnode[11)] was able to obtain some antituberculous substances from rabbit's lungs. As they were soluble in alcohol and acetone they were assumed to be probably fatty acid.

Franc[12)] investigated antituberculous factors in milk. The active substance could pass through a cellophane membrane. It was fractionated by using active charcoal, cation exchange resin, or organic solvents. He assumed that the active fraction was a sort of fatty acid.

Björnesjö[13)-17)] obtained an antituberculous factor from human urine. The active substance was heat stable, dialysable by cellophne membrane, adsorbable on charcoal but not adsorbable on cation exchange resin Dowex 50, and only very slightly soluble in organic solvents. From these facts, he assumed that the tuberculostatic substances in human urine might be some organic acids other than ascorbic acid.

As described above, these investigators obtained various antituberculous factors in different ways from different organs. In short, it is assumed that antimycobacterial factors in animal body fluid may be peptides, amines, (organic bases) or organic acids (fatty acid or not). There, however, has been no systematic investigation in which antituberculous agents have been systematically examined in all organs.

The idea of using ion exchange resins to purify the active factor from the crude materials of organ extracts, serum or urine, have previously been reported by a few investigators[2)16)]. But the systematic fractionation procedure by useng cation and anion exchange resins for isolating antituberculous agents from animal body fluid was first tried by our associates[1)]. Using this fractiona-

tion procedure, the crude materials were separated into four fractions. In each fraction, antituberculous activity was examined. And it was ascertained that the active factors in human urine or rabbit's serum were contained in Fractions I and III (Oshima, Fujita). These active fractions in the serum or urine were very similar to the fractions found in this present investigation dealing with organ extracts, from the following points of view, heat stability, attitude against hydrolysis, solubility in organic solvents, adsorbability in ion exchange resins. From this point of similarity, Fujita[3] considered that these tuberculostatic substances might be made first in tissue cells, and then transported by circulating blood or serum and then to the urine.

There are, however, some differences between the antituberculous substances in organ extracts and these in the serum or human urine. The former are more complicated than the latter. That is, while Fractions I and III of the muscle and the lung are active, Fraction III of the liver and the kidney are not active. It may be worthy of note that Fraction IV of the liver (sugars) is inhibiting to bacilli.

Fraction III, organic acids as antituberculous factors, may not be ascorbic acid or fatty acids because of the physicochemical property described above. Oshima[19] obtained active organic acid as crystals from human urine, but has not yet identified them. The active organic acids of organ extracts may be similar to those in urine, but the identification has not been accomplished at this stage.

Fraction II of all organs has no antituberculous activity. As one of the reasons, it may be pointed out that the absolute amount of active substances contained is small. Anyway, Fraction II cannot inhibit growth of tubercle bacilli even at an eight-fold concentration of the original organ, so it appears that there is no significant relationship between the mechanism of the resistance of the animal body to tuberculous infection and Fraction II.

Fraction I, the fraction of peptides or amino acids, commonly has antituberculous activity not only in each organ but also in the serum and urine. Therefore, it seems that this fraction may play the most important role in the antituberculous mechanism of low molecular substances in animal body fluid. As one of the antituberculous factors of peptides in organ extracts, Dubos found an antituberculous substance in the form of peptide from calf thymus, and called it "thymus peptide". However, in this present investigation, from the fact that tuberculostatic factors in Fraction I could not easily be decreased by hydrolysis, it may be assumed that the active factors in this fraction are not peptides or are peptides which are very resistant to hydrolysis. On the other hand, since the tuberculostatic activity of each amino acid has been denied by other investigators[13][18], it is hard to assume that the tuberculostatic activity of

Fraction I is related to its content of free amino acids. The chemical structure must be thoroughly investigated later.

Sometimes Fraction I of muscle or Fraction IV of the liver showed no tuberculostatic activity even at an eight-fold concentration of the original organs. On the other hand, the crude materials of these two organs have the strongest activity of any of the organs. Therefore, Fraction III may be the most important substance in muscle, and Fraction I in the liver. Fraction I in the muscle and Fraction IV in the liver may play no significant role in tuberculostatic activity.

We observed in Part I, that the difference in antituberculous activity in each organ was related to its content of active substances. And in the present investigation, we observed a lack of uniformity in the activity of fractions according to organs as follows ;

(1) While Fraction I ordinarily showed an apparent activity in each organ, in the muscle a few cases were noted in which no activity was detected in Fraction I. (2) Fraction III in the muscle and the lung always showed activity but not in the liver and the kidney. (3) Fraction IV had no activity in the muscle, the kidney and the lung, but sometimes the activity was noted in the liver. This means that there is a qualitative complicacy in each organ fraction rather than simply a quantitative difference formed in the crude materials. In conclusion, antituberculous agents of each organ extract lack uniformity not only quantitatively but also qualitatively. Thus, although it may be difficult to determine clearly, this lack of uniformity may be concerned to some extent organ disposition to tuberculous infection. And it cannot be negated that the presence of such antituberculous substances in animal organs may play some role in the mechanism of resistance of each organ to tuberculous infection. Therefore, to discover the really active substance, further investigations must be continued.

SUMMARY

1) Extracts from the muscle, the liver, the kidney and the lung of rabbits were fractionated by using systematically the ion exchange resins (Amberlite IR-120 and IRA-400) into four fractions, that is, Fraction I ; amino acid or peptide, Fraction II ; organic base, Fraction III ; organic acid, Fraction IV ; sugar.

2) The fractions which have tuberculostatic activity, more or less, were as follows ; Fraction I in these four organs, Fraction III in muscle and lung, Fraction IV in liver.

3) The active fraction (muscle, liver) acted more inhibitingly on the growth of bovine type tubercle bacilli and less on the avian type, than on the human type.

4) The tuberculo-inhibitory action of the active factors was thought to be bactericidal rather than bacteriostatic.

5) The tuberculostatic activity of each active fraction was not increased by immunization.

6) Chemical examinations of each fraction were carried out by using chromatography, but identification of each component could not be finished.

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